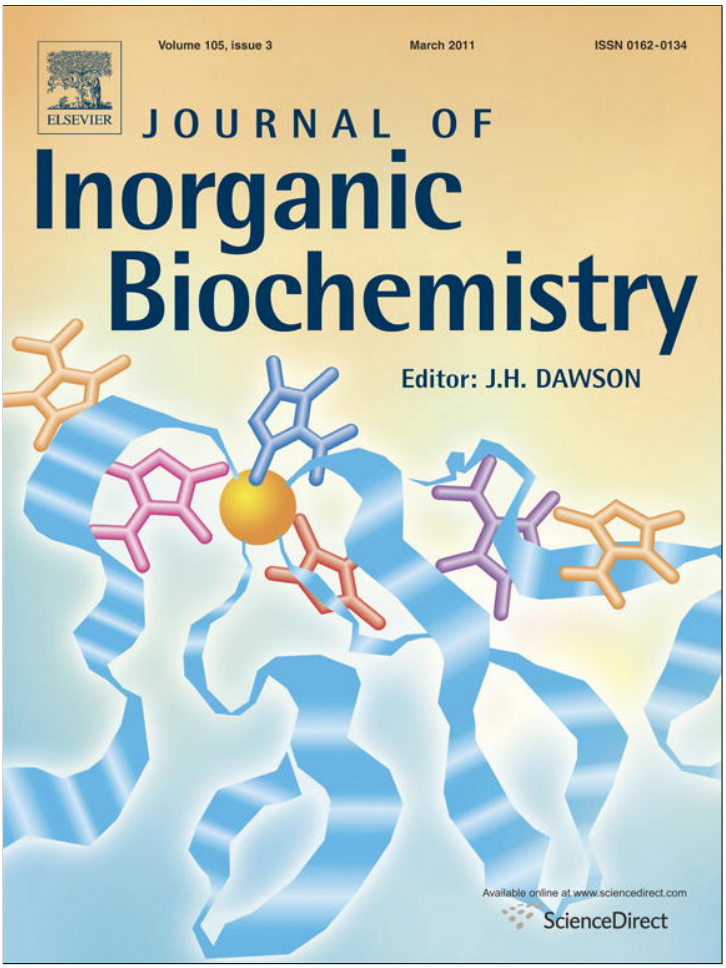


Journal of Inorganic Biochemistry 105 (2011) 420–425

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Selective recognition of homocysteine and cysteine based on new ruthenium(II) complexes

Mei-Jin Li *, Chong-Qing Zhan, Mei-Juan Nie, Guo-Nan Chen, Xi Chen *

Key Laboratory of Analysis and Detection Technology for Food Safety, Ministry of Education, Fujian Provincial Key Laboratory of Analysis & Detection Technology for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou 350002, China

ARTICLE INFO

Article history:
Received 30 August 2010
Received in revised form 12 December 2010
Accepted 15 December 2010
Available online 25 December 2010

Keywords:
Ruthenium(II) complexes
Homocysteine
Cysteine
Aldehyde
Luminescence

ABSTRACT

A series of the new ruthenium(II) complexes with different number of aldehyde groups have been synthesized and characterized for the simple and selective sensing of homocysteine (Hcy) and cysteine (Cys). The reaction of these ruthenium(II) complexes with Hcy and Cys afforded thiazinane or thiazolidine derivatives which resulted in the obvious changes in the UV–visible spectra and strong enhancement of the luminescence intensity of the system. The luminescence enhancement of $[Ru(dmb)_2(L2)]^{2+}$ (dmb: 4,4′-dimethyl-2,2′-bipyridine) showed a good linearity in the concentration of 4.2–350 μM and 6–385 μM with the detection limits of 0.3 μM and 1 μM for Hcy and Cys, respectively. The absorption and emission bands from metal-to-ligand charge transfer transition in the visible region and the large Stokes shift of the ruthenium(II) complex chromophore made it suitable for biological applications.

1. Introduction

Homocysteine (Hcy) and cysteine (Cys) are important amino acids containing a free thiol moiety in living systems, and they plays a critical role in a variety of cellular functions, such as detoxification and metabolism [1,2]. It was discovered that there is a link between the levels of Hcy or Cys and various types of vascular and renal diseases [3–5]. Hcy is a risk factor for Alzheimer's [6] and cardiovascular diseases [7] at elevated levels in plasma while the deficiency of Cys is associated with slowed growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness [8]. The determination of specific thiols is often based on redox chemistry or derivatization with chromophores or fluorophores in conjunction with HPLC or capillary electrophoresis separations or via immunoassays [9].

Recently, some organic chromophores were developed as chemodosimeters for Hcy and Cys [10,11]. These chromophores by reaction of aldehyde groups with Hcy and Cys had been designed and applied in many fields [12,13]. However, these chromophores are mainly limited to organic fluorophores suffering from undesirability such as low photostability, high background fluorescence, the UV excitation and emission wavelengths and the small Stokes shift. In recent years, increasing attention has been paid to design the phosphorescent metal complexes as chemosensors for Hcy and Cys [14–19] because of advantageous photophysical properties of transition metal complexes such as high stabilities and relatively long lifetimes compared with those of organic fluorophores. Besides, metal complexes can easily be modified with different functional groups to tune the energy band. Of all d^6 transition metal complexes, ruthenium(II) polypyridine complexes are amongst the earliest and most widely studied systems due to their stabilities and rich photochemical and photophysical properties associated with metal-to-ligand charge-transfer (MLCT) transition [20–24]. Although there were a number of reports on the luminescent sensing of anions [25–28], metal cations [29–33] and molecular oxygen [34] based on the ruthenium(II) polypyridine probes, the ruthenium(II)-bipyridine complexes that can be directly applied to probe the biomolecules have rarely been reported [35–40]. In our paper, a series of new ruthenium(II) complexes containing aldehyde groups (Scheme 1) were synthesized and characterized to recognize Hcy and Cys by the formation of thiazinane or thiazolidine (Scheme 2). A strong luminescence response was found upon reaction of the ruthenium(II) chromophore with Hcy or Cys only, but not with other amino acids, indicating a high specificity for recognition of Hcy and Cys. Some electron-donating groups (CH_3) were also modified in chromophore which was expected to improve the recognition of Hcy and Cys.

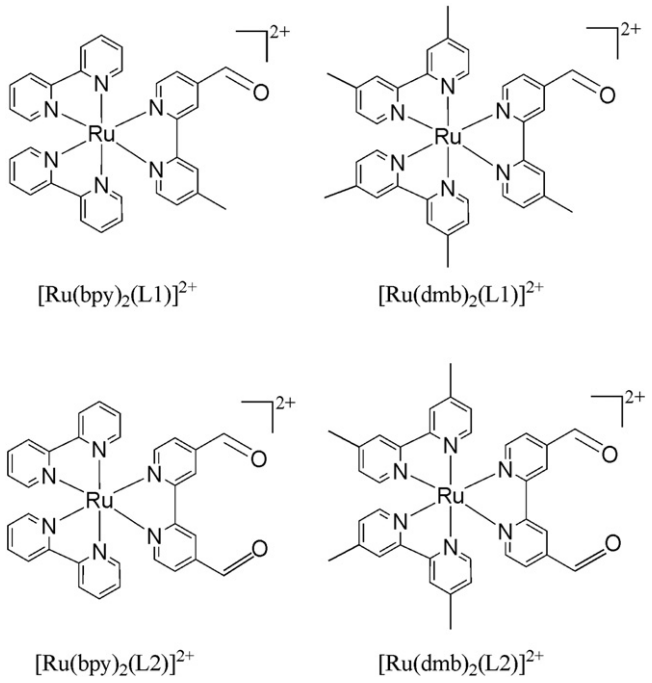
2. Experimental

2.1. Reagents and instrumentation

$RuCl_3$, NH_4PF_6, 2,2′-bipyridine (bpy), 4,4′-dimethyl-2,2′-bipyridine (dmb), cysteine, homocysteine and other amino acids were purchased from Sigma-Aldrich. Other chemicals were analytical reagent graded and used as received. *cis*-$Ru(bpy)_2Cl_2 \cdot xH_2O$ [41], *cis*-$Ru(dmb)_2Cl_2 \cdot xH_2O$ [41], 4-methyl-2,2′-bipyridine-4′-carboxaldehyde (L1) [42] and 4,4′-diformyl-

* Corresponding authors.
E-mail addresses: mjli@fzu.edu.cn (M.-J. Li), xichen@xmu.edu.cn (X. Chen).

0162-0134/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2010.12.007



Scheme 1. Structures of the synthesized ruthenium(II) complexes.

2,2′-bipyridine (L2) [43] were prepared according to the literature method. All solutions were prepared with deionized water (Milli-Q, Millipore). The pH of the Tris–HCl buffer solution was adjusted with HCl. Emission spectra were recorded on a HITACHI F-4600 fluorescence spectrophotometer. A 1.00 cm path length rectangular quartz cell was used for all emission measurements. ^{1}H NMR spectra were recorded on a Bruker DPX-300 Fourier Transform NMR spectrometer with chemical shifts reported relative to tetramethylsilane. Positive-ion fast atom bombardment (FAB) and electron impact (EI) mass spectra (MS) were recorded on a Finnigan MAT95 mass spectrometer. Positive ion electrospray ionization (ESI) mass spectra were recorded on a Finnigan LCQ spectrometer. Elemental analysis of the complexes was performed on a Carlo Erba 1106 elemental analyzer.

2.2. Synthesis of complexes

$[Ru(bpy)_2(L1)](PF_6)_2\cdot H_2O$ was prepared by modification of a literature method for $[Ru(bpy)_2(phen)]^{2+}$ (phen: 1,10-phenanthroline) [44]. To a solution of *cis*-$Ru(bpy)_2Cl_2\cdot xH_2O$ (50 mg, 0.10 mmol) in absolute ethanol (50 mL) was added L1 (24 mg, 0.12 mmol), and the mixture was heated to reflux under N_2 for 6 h, during which the purple black solution turned red brown. After removal of the solvent under reduced pressure, the residue in the form of chloride salt was dissolved in a minimum amount of water, and metathesis reaction upon the addition of a saturated methanolic solution of NH_4PF_6 afforded the desired complex as a red-orange solid, which was then obtained by filtration, and subsequent recrystallization by vapor diffusion of diethyl ether into acetonitrile solutions of the complexes gave $[Ru(bpy)(L1)](PF_6)_2\cdot H_2O$ as red crystals. Yield: 47 mg, 50%. Positive FAB-MS: *m/z* 611, calc. for $C_{32}H_{25}N_6ORu$ 611.12 ($[M–HPF_6–PF_6]^+$). Anal. Calc. for $C_{32}H_{26}N_6F_{12}P_2ORu\cdot H_2O$ (%): C, 41.78; H, 3.05; N, 9.14. Found (%): C, 41.52; H, 3.21; N, 9.37. ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): 10.18 (s (singlet), 1H, bpy-CHO), 8.85 (s, 1H, bpy), 8.54 (t (triplet), J = 8.1 Hz, 5H, bpy), 8.10 (m (multiplet), 4H, bpy), 8.03(d (doublet), J = 5.7 Hz, 1H, bpy), 7.73 (m, 5H, bpy), 7.59 (d, J = 5.7 Hz,1H, bpy), 7.42 (m, 4H, bpy), 7.32 (d, J = 4.9 Hz,1H, bpy), 2.59 (s, 3H, CH_3).

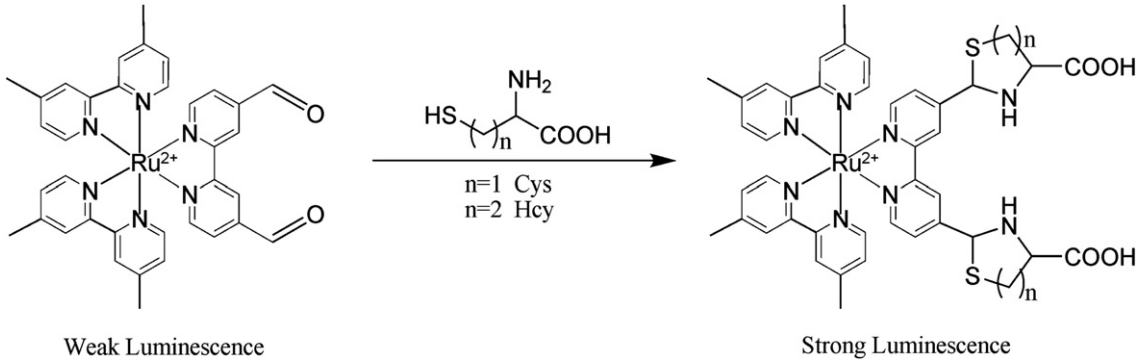
$[Ru(bpy)_2(L2)](PF_6)_2\cdot H_2O$ was prepared using a procedure similar to that for $[Ru(bpy)_2(L1)](PF_6)_2$ except L2 was used instead of L1. Recrystallization by vapor diffusion of diethyl ether into acetonitrile solutions of the complexes gave $[Ru(bpy)_2(L2)](PF_6)_2$ as black crystals. Yield: 44 mg, 45%. Positive FAB-MS: *m/z* 626, calc. for $C_{32}H_{23}N_6O_2Ru$ 625.12 ($[M–HPF_6–PF_6]^+$). Anal. Calc. for $C_{32}H_{24}N_6F_{12}P_2O_2Ru\cdot H_2O$ (%): C, 41.16; H, 2.79; N, 9.00. Found (%): C, 41.37; H, 2.65; N, 9.37. ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): 10.21 (s, 2H, bpy-CHO), 9.04 (s, 2H, bpy), 8.54 (m, 4H, bpy), 8.10 (m, 6H, bpy), 7.82(d,J = 1.8 Hz, 1H, bpy), 7.80 (d, J = 1.8 Hz, 1H, bpy), 7.73 (d, J = 5.1 Hz, 2H, bpy), 7.68 (d, J = 5.6 Hz, 2H, bpy), 7.44 (m, 4H, bpy).

$[Ru(dmb)_2(L1)](PF_6)_2\cdot H_2O$ was prepared using a procedure similar to that for $[Ru(bpy)_2(L1)](PF_6)_2$ except *cis*-$Ru(dmb)_2Cl_2\cdot xH_2O$ was used instead of *cis*-$Ru(bpy)_2Cl_2\cdot xH_2O$. Recrystallization by vapor diffusion of diethyl ether into acetonitrile solutions of the complexes gave $[Ru(dmb)_2(L1)](PF_6)_2$ as black crystals. Yield: 46 mg, 42%. Positive FAB-MS: *m/z* 812, 667, calc. for $C_{36}H_{34}N_6F_6PORu$ 812.73 ($[M\text{-}PF_6]^+$) and $C_{36}H_{33}N_6ORu$ 667.18 ($[M–HPF_6–PF_6]^+$). Anal. Calc. for $C_{36}H_{34}N_6F_{12}P_2ORu\cdot H_2O$ (%): C, 44.31; H, 3.69; N, 8.62. Found (%): C, 44.27; H, 3.31; N, 8.60. ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): 10.18 (s, 1H, bpy-CHO), 8.83 (s, 1H, bpy), 8.54 (s, 1H, bpy), 8.37 (s, 4H, bpy), 8.03 (d, J = 5.6 Hz, 1.5, 1H, bpy), 7.72 (d, J = 4.7 Hz, 1H, bpy), 7.58 (d, J = 5.8 Hz, 1H, bpy), 7.52 (m, 4H, bpy), 7.31 (d, J = 4.8 Hz, 1H, bpy), 7.23 (m, 4H, bpy), 2.56 (m, 15H, bpy).

$[Ru(dmb)_2(L2)](PF_6)_2$ was prepared using a procedure similar to that for $[Ru(dmb)_2(L1)](PF_6)_2$ except L2 was used instead of L1. Recrystallization by vapor diffusion of diethyl ether into acetonitrile solutions of the complexes gave $[Ru(dmb)_2(L2)](PF_6)_2$ as black crystals. Yield: 45 mg, 40%. Positive FAB-MS: *m/z* 681, calc. for $C_{36}H_{31}N_6O_2Ru$ 681.16 ($[M–HPF_6–PF_6]^+$). Anal. Calc. for $C_{36}H_{32}N_6F_{12}P_2O_2Ru\cdot H_2O$ (%): C, 43.68; H, 3.43; N, 8.49. Found (%): C, 43.57; H, 3.56; N, 8.09. ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): 10.12 (s, 2H, bpy-CHO), 8.92 (s, 2H, bpy), 8.28 (d, J = 6.6 Hz, 4H, bpy), 7.96 (d, J = 5.7 Hz, 2H, bpy),7.69(d, J = 4.8 Hz, 2H, bpy), 7.42 (d, J = 5.7 Hz, 2H, bpy), 7.36 (d, J = 5.6 Hz, 2H, bpy), 7.20 (d, J = 5.9 Hz, 2H, bpy), 7.12 (d, J = 4.9 Hz, 2H, bpy), 2.45(d, J = 11.3 Hz, 12H, bpy).

3. Methods

Amino acid titration of the complexes was performed in acetonitrile–Tris buffer (50 mM, pH = 7.2, 10:1 v/v) solution. The



Scheme 2. The recognition mechanism of $[Ru(dmb)_2(L2)](PF_6)_2$for Hcy and Cys.

samples containing different concentration of Hcy or Cys were mixed for 10 min before the UV–visible (UV–vis) absorption and photoluminescence spectra were recorded.

4. Results and discussion

4.1. Absorption and photoluminescence properties

The electronic absorption spectra of complexes in acetonitrile were mainly dominated by intense high-energy absorption bands at *ca.* 276–308 nm, and weak low-energy bands at *ca.* 416–492 nm, typical of ruthenium(II) tris(bipyridine) derivatives [45]. With reference to previous studies on the related ruthenium(II) polypyridine systems [24], the higher energy absorption bands were assigned as intraligand (IL) transition, while the low-energy bands were assigned to the metal-to-ligand charge transfer (MLCT, dπ(Ru) → π*(ligand)) transitions, which were absent in the electronic absorption spectra of the free ligands. The ruthenium(II) complexes were found to emit weakly with emission maxima at *ca.* 605–617 nm at room temperature in acetonitrile solution, assigned as derived from a triplet MLCT state, similar to that observed in other related ruthenium(II) diimine systems. The luminescence quantum yield of the complexes with two aldehyde groups in air-equilibrated acetonitrile solution was a little lower than that of the complexes with one aldehyde group. The weak emission of the new ruthenium(II) complexes could be explained by the strong electron-withdrawing property of aldehyde group in the ligand that quenches the MLCT luminescence of the complexes. The photophysical data of the new ruthenium(II) complexes were listed in Table 1. As shown in Table 1, substitution of bpy with electron-donating group (CH_3) would result in the slight red shift of the emission maxima. This phenomena could be explained that the electron-donating group would destabilize the HOMO (dπ(Ru)) and result in the smaller energy gap between HOMO (dπ(Ru)) and the LUMO (π*(L-CHO)) and hence decrease the emission energy.

Upon addition of Hcy to the acetonitrile–Tris buffer solution of [Ru(dmb)$_2$(L2)](PF$_6$)$_2$, obvious changes were observed in the absorption spectra, especially in the MLCT absorption band as shown in Fig. 1. With the interaction between Hcy and [Ru(dmb)$_2$(L2)](PF$_6$)$_2$, the MLCT absorption band at 491 nm was found to show a blue shift to 460 nm and its absorption intensity increased to some degree. These phenomena indicated that a new product was formed in the presence of Hcy. Addition of Cys to the acetonitrile–Tris buffer solution of ruthenium(II) complexes also resulted in large changes in the absorption spectra. According to the reported literature [10], upon interaction of Hcy or Cys, [Ru(dmb)$_2$(L2)](PF$_6$)$_2$ with electron-withdrawing groups (aldehyde) would form thiazinane or thiazolidine to destroy the electron-withdrawing ability of aldehydes, which destabilized the π* orbital of ligand((L2)) and then resulted in the larger energy gap between dπ(Ru) and π*(L2) and the blue shift of the MLCT absorption band. The formation of thiazinane or thiazolidine also led to some changes in LC (ligand-centered) absorption bands. After the addition of 100 equivalent Hcy to the acetonitrile–Tris buffer solution of [Ru(dmb)$_2$(L2)](PF$_6$)$_2$, the emission intensity was enhanced by about 10 fold in the luminescence spectra (Fig. 1c). While the same amount of Cys resulted in a weaker enhancement of emission intensity (Fig. 1b). These spectral changes were mainly ascribed to the formation of thiazinane or thiazolidine which improved the electron-donating ability of the ligand and resulted in the enhancement of the luminescence of the probes.

Table 1
Photophysical data of the new ruthenium(II) complexes.

Complex	Absorption λ_{abs}, nm (ε, M^{-1} cm^{-1})	Φ ($\Phi_{ref}=1$)	Emission λ_{em}, nm
[Ru(bpy)$_2$(L1)]$^{2+}$	280 (94460), 420 (17330), 452 (20435)	0.26	605
[Ru(bpy)$_2$(L2)]$^{2+}$	280 (13310), 304 (62445), 418 (27180), 481 (24680)	0.15	607
[Ru(dmb)$_2$(L1)]$^{2+}$	276 (45020), 428 (8825), 472(9665)	0.18	612
[Ru(dmb)$_2$(L2)]$^{2+}$	276 (52095), 308(23460), 416(13475), 492(11150)	0.14	617

$Ru(bpy)_3Cl_2$ in air-equilibrated acetonitrile solution was used as reference (Φ = 1.0, ex: 450 nm).

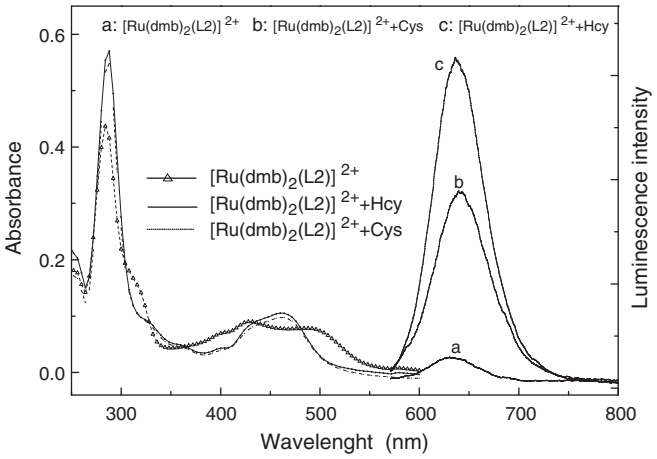


Fig. 1. Electronic absorption and luminescence spectra of [Ru(dmb)$_2$(L2)](PF$_6$)$_2$ (7.0×10^{-6} M) in the absence and presence of Hcy or Cys (100 equiv) in acetonitrile–Tris buffer solution (pH = 7.2, 50 mM, 10:1, v/v).

4.2. UV–vis absorption titration of the ruthenium(II) complexes with Hcy and Cys

Upon addition of Hcy or Cys to the acetonitrile–Tris buffer solution of the new ruthenium(II) complexes, spectral changes in the both IL transition bands and MLCT bands were observed. Upon addition of Hcy or Cys to [Ru(bpy)$_2$(L1)](PF$_6$)$_2$ and [Ru(dmb)$_2$(L1)](PF$_6$)$_2$ with one aldehyde group in acetonitrile–Tris buffer solution, the slight decrease of the MLCT band at about 490 nm and the increase of the band at 455 nm produced a perfectly clean isosbestic point at *ca.* 465 nm. While addition of Hcy or Cys to [Ru(bpy)$_2$(L2)](PF$_6$)$_2$ and [Ru(dmb)$_2$(L2)](PF$_6$)$_2$ with two aldehyde groups, there were significant changes in the UV–vis spectra, especially in the MLCT bands. The UV–vis spectral traces of [Ru(dmb)$_2$(L2)](PF$_6$)$_2$ upon addition of Hcy is shown in Fig. 2, in which well-defined isosbestic points were observed. As shown in Fig. 2, the MLCT absorption band at 492 nm decreased in intensity and a new absorption band at 461 nm was formed with a blue-shift of 31 nm and two clear isosbestic points at 431 and

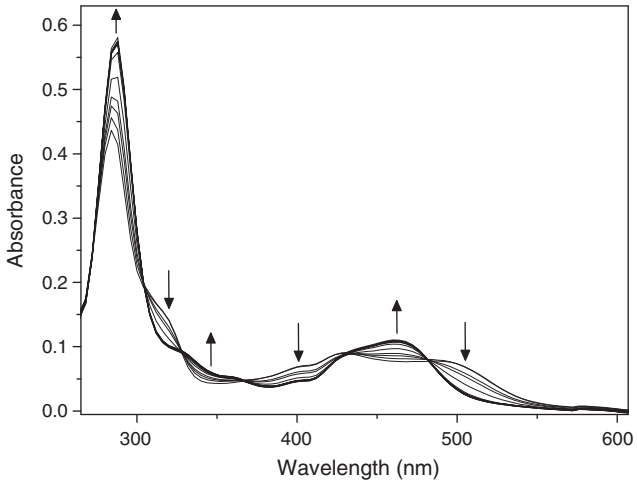


Fig. 2. UV–vis absorption spectra of [Ru(dmb)$_2$(L2)](PF$_6$)$_2$ (7.0×10^{-6} M) in acetonitrile–Tris buffer solution (pH = 7.2, 50 mM, 10:1, v/v) upon treatment with various amounts of Hcy.

482 nm. The absorption bands at 287, 318, 344 and 400 nm also showed some changes with isosbestic points at 304, 328 and 366 nm. All the results suggested the formation of thiazinane in the aldehyde groups of ruthenium(II) complex would reduce the electron-withdrawing effect of the ligand and result in the large changes in the UV–vis absorption spectra with a blue-shift of MLCT band. The studies also showed that more aldehyde groups in the ruthenium(II) complexes would cause larger spectral changes in the UV–vis spectra upon addition of Hcy, indicating that aldehyde groups definitely play a key role in the recognition of Hcy and Cys. The spectral changes of other ruthenium(II) complexes upon addition of Hcy or Cys were listed in the supporting information.

4.3. Luminescence titration of the ruthenium(II) complexes with Hcy and Cys

The recognition ability of the new ruthenium(II) complexes for Hcy and Cys was also investigated by emission spectrophotometric studies. The emission intensity of complexes was strongly enhanced upon the addition of Hcy or Cys with a small red shift of emission maxima. Upon addition of Hcy to the $[Ru(bpy)_2(L1)](PF_6)_2$ and $[Ru(dmb)_2(L1)](PF_6)_2$ acetonitrile–Tris buffer solution, the emission intensity was only enhanced by about 1.6 and 1.9 fold, respectively. While the emission intensity of $[Ru(bpy)_2(L2)](PF_6)_2$ was enhanced by about 8.5 fold after addition of Hcy and the emission intensity of $[Ru(dmb)_2(L2)](PF_6)_2$ was enhanced most strongly by about 10.1-fold upon addition of Hcy (Fig. 3). Such strong enhancement of the emission intensity for $[Ru(bpy)_2(L2)](PF_6)_2$ and $[Ru(dmb)_2(L2)](PF_6)_2$ could be explained by the high sensitivity of two aldehyde groups in the complexes. All results demonstrated that substitution with electron-donating group (CH_3) on the bpy and more aldehyde groups in the complexes would cause larger spectral changes in the emission spectra. In the case of Cys, the emission intensity of complexes $[Ru(bpy)_2(L1)](PF_6)_2$, $[Ru(bpy)_2(L2)](PF_6)_2$, $[Ru(dmb)_2(L1)](PF_6)_2$ and $[Ru(dmb)_2(L2)](PF_6)_2$ was enhanced by about 1.3-fold, 3.5-fold, 1.6-fold and 4.5-fold, respectively. All the complexes were more sensitive toward Hcy than Cys, probably due to the easier formation of thiazinane. These results were in agreement with the observed trends in the studies using the UV–vis spectrophotometric method, in which complex $[Ru(dmb)_2(L2)](PF_6)_2$ and $[Ru(bpy)_2(L2)](PF_6)_2$ with two aldehyde groups gave stronger affinity toward Hcy and Cys.

The emission spectral changes of $[Ru(dmb)_2(L2)](PF_6)_2$ upon addition of Hcy and the changes of the emission intensity as a function of the added Hcy concentration are shown in Fig. 3. With the increase of the Hcy concentration, the luminescence intensity of the solution was gradually

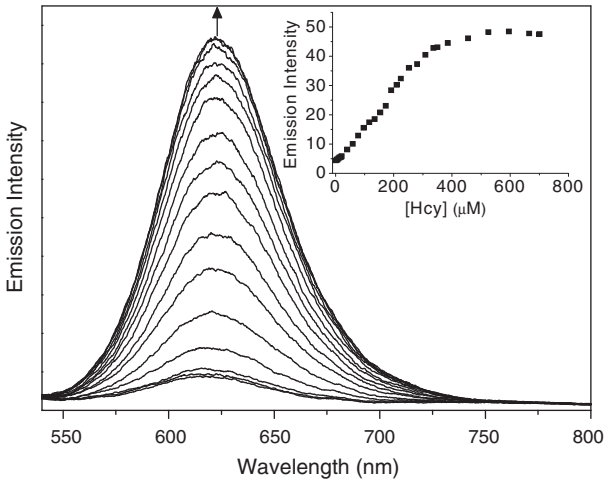


Fig. 3. Emission spectral traces of $[Ru(dmb)_2(L2)](PF_6)_2$ (7.0×10^{-6} M) in acetonitrile–Tris buffer solution (pH = 7.2, 50 mM, 10:1, v/v) upon addition of Hcy. Excitation at isosbestic point: 482 nm. Inset shows the emission intensity at 622 nm as a function of the added Hcy concentration.

increased. The luminescence enhancement of $[Ru(dmb)_2(L2)]^{2+}$ showed a good linearity in the concentration of 4.2–350 μM with a detection limit of 0.3 μM for Hcy. Similarly, the luminescence enhancement of $[Ru(dmb)_2(L2)]^{2+}$ showed a good linearity in the concentration and 6.0–385 μM with a detection limit of 1.0 μM for Cys. The detection limits of other ruthenium(II) complexes for Hcy and Cys were listed in Table 2. The results further confirmed that the more aldehyde groups in the complex would make it better sensitive toward Hcy and Cys, especially for Hcy. Substitution with electron-donating group (CH_3) on the bpy would also improve the recognition ability of complexes for Hcy and Cys. Based on these results, our effort was made to design new probes for discriminating Hcy and Cys, and this is still in progress.

4.4. Reaction of the ruthenium(II) complexes with Hcy or Cys

The reaction of the ruthenium(II) complexes with Hcy and Cys was also confirmed by positive-ion ESI-MS experiments. The 1:1 adduct, $[Ru(dmb)_2(L1\text{-Hcy})]^{2+}$, $[Ru(dmb)_2(L1\text{-Cys})]^{2+}$, and the 1:2 adduct, $[Ru(dmb)_2(L2\text{-2Hcy})]^{2+}$, $[Ru(dmb)_2(L2\text{-2Cys})]^{2+}$ were observed as ion clusters at *m/z* 392.8, 386.3, 458.2 and 444.8, respectively, in the positive-ion ESI-mass spectrum of an acetonitrile-H_2O solution (7:3, v/v) of $[Ru(dmb)_2(L1)](PF_6)_2$ or $[Ru(dmb)_2(L2)](PF_6)_2$ and Hcy or Cys. The ESI-mass spectra are listed in the supporting information. Attempts have been made to further establish the importance of the aldehyde moiety in the recognition studies by the Job's plotting analysis. Job's plot for the complexation of $[Ru(dmb)_2(L1)](PF_6)_2$ and $[Ru(dmb)_2(L2)](PF_6)_2$ for Hcy also showed 1:1 and 1:2 reaction stoichiometry as shown in Fig. 4. The results demonstrated that all aldehyde groups in the complexes would react with Hcy or Cys. ^{1}H NMR spectroscopy of $[Ru(dmb)_2(L1)](PF_6)_2$ and Hcy in CD_3CN:D_2O (10:1) was also carried out to confirm the reaction of the ruthenium(II) complexes with Hcy. Upon addition of Hcy, the signal at 10.14 ppm corresponding to CHO was weakened, and the new signal corresponding to NCHS appeared at 5.44 ppm as shown in Fig. S8 (Supporting Information). The results further demonstrated that Hcy could conjugate with the aldehyde group of the ruthenium(II) complexes.

4.5. Reaction of the ruthenium(II) complexes with other amino acids

The selectivity of new probes for Hcy and Cys was also studied. Photoluminescence properties of ruthenium(II) complexes in acetonitrile–Tris buffer (10:1, v/v) were also investigated upon addition of other amino acids (alanine, aminobutyric acid, arginine, asparagine, glutamine, glycine, histdine, hydroxy-proline, leucine, lysine, methionine, ornithine, phenylalanine, sarcosine, serine, threonine, tryptophane, tyrosine, valine, and glutathione). No obvious or very small luminescence increase was observed upon addition of other amino acids compared with Hcy and Cys, indicating that the formation of thiazinane and thiazolidine was a key role for the selective recognition of Hcy and Cys. The changes of luminescence upon addition of various amino acids at the same concentration to $[Ru(dmb)(L2)](PF_6)_2$ are presented in Fig. 5. The selectivity of other complexes toward Hcy and Cys was shown in the supporting information.

5. Conclusion

A series of ruthenium(II) complexes with different number of aldehyde groups had been synthesized for the simple and selective determination of Hcy and Cys. The reaction of these ruthenium(II)

Table 2
The detection limits of the ruthenium(II) complexes (7.0×10^{-6} M) for Hcy or Cys.

Complex / Amino acid	$[Ru(bpy)_2(L1)]^{2+}$	$[Ru(bpy)_2(L2)]^{2+}$	$[Ru(dmb)_2(L1)]^{2+}$	$[Ru(dmb)_2(L2)]^{2+}$
Hcy (M)	4×10^{-6}	5×10^{-7}	2×10^{-6}	3×10^{-7}
Cys (M)	5×10^{-6}	2×10^{-6}	4×10^{-6}	1×10^{-6}

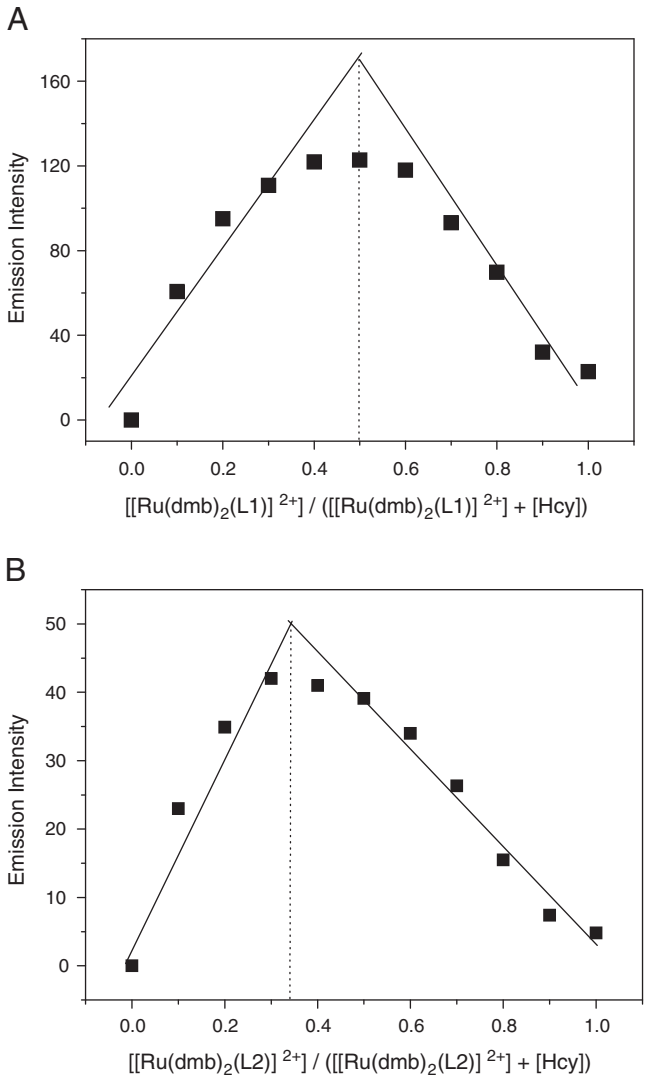


Fig. 4. Job's plots for the reactions of $[Ru(dmb)_2(L1)](PF_6)_2$ (A) and $[Ru(dmb)_2(L2)](PF_6)_2$ (B) with Hcy in the acetonitrile–Tris buffer solution.

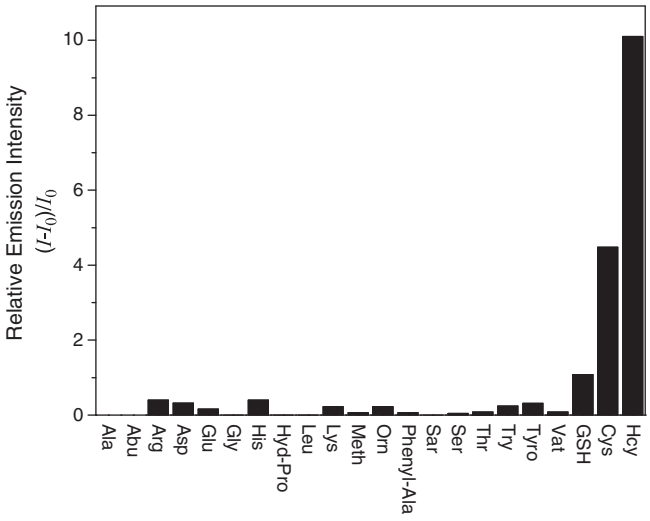


Fig. 5. Luminescence intensity increase $(I-I_o)/I_o$ of $[Ru(dmb)_2(L2)](PF_6)_2$ (7.0×10^{-6} M) in acetonitrile–Tris buffer solution (pH = 7.2, 50 mM, 10:1, v/v) upon the addition of the 100 equivalent Hcy, Cys and other amino acids.

complex with Hcy and Cys afforded stable derivatives thiazinanes or thiazolidines to enhance the luminescence intensity of system. The method for the recognition of Hcy and Cys is selective and sensitive without the interference of other amino acids. Additionally, the ruthenium(II) complex chromophore with more aldehyde groups and electron-donating groups (CH_3) could improve the sensitivity of the complexes toward Hcy and Cys. Compared to the reported probes for the detection of Hcy and Cys, there are several advantageous properties for this kind of ruthenium(II) complex probe such as visible-light excitation and emission wavelengths with a larger Stokes shift, a remarkable change of the emission, and high selectivity and sensitivity. This work provided a new strategy for the design of other transition metal complex-based luminescence probes for selective recognition of Hcy or Cys and could be expected to extend the applications of transition complexes in various biological sensing.

Abbreviations

Hcy	homocysteine
Cys	cysteine
IL	intraligand
LC	ligand-centered
MLCT	metal-to-ligand charge transfer
bpy	2,2′-bipyridine
dmb	4,4′-dimethyl-2,2′-bipyridine
L1	4-methyl-2,2′-bipyridine-4′-carboxaldehyde
L2	4,4′-diformyl-2,2′-bipyridine
phen	1,10-phenanthroline
HOMO	highest occupied molecular orbital
LUMO	lowest unoccupied molecular orbital
FAB	fast atom bombardment
EI	electron impact
ESI	electrospray ionization

Acknowledgment

This work was supported by the National Scientific Foundation of China (NSFC No. 20801014), Scientific Foundation of Fujian Province (No. 2009J05026) and the funding from the Fuzhou University (No. 826505, 2008-XQ-08).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jinorgbio.2010.12.007.

References

[1] R. Carmel, D.W. Jacobsen (Eds.), Homocysteine in Health and Disease, Cambridge University Press, Cambridge, U.K, 2001.
[2] Z.A. Wood, E. Schroder, J.R. Harris, L.B. Poole, Trends Biochem. Sci. 28 (2003) 32–40.
[3] B. Fowler, Kidney Int. 59 (2001) S221–S229.
[4] B.A. Brown, J.L. Marx, T.P. Ward, R.D. Hollifield, J.S. Dick, J.J. Brozetti, R.S. Howard, A.B. Thach, Ophthalmology 109 (2002) 28–290.
[5] C.V. Guldener, F. Stam, C.D.A. Stehouwer, Kidney Int. 59 (2001) S234–S237.
[6] S. Seshadri, A. Beiser, J. Selhub, P.F. Jacques, I.H. Rosenberg, R.B. D'Agostino, P.W.F. Wilson, New Engl. J. Med. 346 (2002) 476–483.
[7] H. Refsum, P.M. Ueland, O. Nygard, S.E. Vollset, Annu. Rev. Med. 49 (1989) 31–62.
[8] S. Shahrokhian, Anal. Chem. 73 (2001) 5972–5978.
[9] O. Nekrassova, N.S. Lawrence, R.G. Compton, Talanta 60 (2003) 1085–1095.
[10] O. Rusin, N.N.S. Luce, R.A. Agbaria, J.O. Escobedo, S. Jiang, I.M. Warner, F.B. Dawan, K. Lian, R.M.J. Strongin, J. Am. Chem. Soc. 126 (2004) 438–439.
[11] W.H. Wang, O. Rusin, X.Y. Xu, K.K. Kim, J.O. Escobedo, S.O. Fakayode, K.A. Fletcher, M. Lowry, C.M. Schowalter, C.M. Lawrence, F.R. Fronczek, I.M. Warner, R.M. Strongin, J. Am. Chem. Soc. 127 (2005) 15949–15958.
[12] T.J. Tolbert, C.H. Wong, Angew. Chem. Int. Ed. 41 (2002) 2171–2174.
[13] A.J.L. Cooper, A. Meinster, J. Biomol. Chem. 257 (1982) 816–826.
[14] M.L. Ho, F.M. Hwang, P.N. Chen, Y.H. Hu, Y.M. Cheng, K.S. Chen, G.H. Lee, Y. Chi, P.T. Chou, Org. Biomol. Chem. 4 (2006) 98–103.

[15] Q. Zhao, T.Y. Cao, F.Y. Li, X.H. Li, H. Jing, T. Yi, C.H. Huang, Organometallics 26 (2007) 2077–2081.
[16] H.L. Chen, Q. Zhao, Y.B. Wu, F.Y. Li, H. Yang, T. Yi, C.H. Huang, Inorg. Chem. 46 (2007) 11075–11081.
[17] Q. Zhao, F.Y. Li, C.H. Huang, Chem. Soc. Rev. (2010), doi:10.1039/b915340c.
[18] K.W. Huang, H. Yang, Z.G. Zhou, H.L. Chen, F.Y. Li, C.H. Huang, Inorg. Chim. Acta 362 (2009) 2577–2580.
[19] S.M. Ji, H.M. Guo, X.L. Yuan, X.H. Li, H.D. Ding, P. Gao, C.X. Zhao, W.T. Wu, W.H. Wu, J.Z. Zhao, Org. Lett. 12 (2010) 2876–2879.
[20] J.P. Paris, W.W. Brandt, J. Am. Chem. Soc. 81 (1959) 5001–5002.
[21] R.J. Watts, J. Chem. Edu. 60 (1983) 834.
[22] E.A. Seddon, K.R. Seddon, The Chemistry of Ruthenium, Elsevier, Amsterdam, 1984.
[23] R.A. Krause, Struct. Bonding 6 (1987) 1–52.
[24] A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Belser, A. Von Zelewsky, Coord. Chem. Rev. 84 (1988) 85–277.
[25] P.D. Beer, F. Szemes, V. Balzani, C. Sala, M. Drew, S. Dent, M. Maestri, J. Am. Chem. Soc. 119 (1997) 11864–11875.
[26] S. Watanabe, O. Onogawa, Y. Komatsu, K. Yoshida, J. Am. Chem. Soc. 120 (1998) 229–230.
[27] J. Anzenbacher, D. Tyson, K. Jursíkova, F. Castellano, J. Am. Chem. Soc. 124 (2002) 6232–6233.
[28] A. Ghosh, B. Ganguly, A. Das, Inorg. Chem. 46 (2007) 9912–9918.
[29] P.D. Beer, O. Kocian, R.J. Mortimer, C. Ridgway, J. Chem. Soc. Chem. Commun. (1991) 1460–1463.
[30] F. Barigelletti, L. Flamigni, G. Calogero, L. Hammarstrom, J.P. Sauvage, J.P. Collin, Chem. Commun. (1998) 2333–2334.
[31] P.D. Beer, Acc. Chem. Res. 31 (1998) 71–80.
[32] V.W.W. Yam, V.W.M. Lee, Inorg. Chem. 36 (1997) 2124–2129.
[33] M.J. Li, B.W.K. Chu, N. Zhu, V.W.W. Yam, Inorg. Chem. 46 (2007) 720–733.
[34] E. Carraway, J. Demas, B. DeGraff, J. Bacon, Anal. Chem. 63 (1991) 337–342.
[35] J. Lakowicz, E. Terpetschnig, Z. Murtaza, H. Szmacinski, J. Fluoresc. 7 (1997) 17–25.
[36] C.F. Chow, B.W. Chiu, M.W. Lam, W.Y. Wong, J. Am. Chem. Soc. 125 (2003) 7802–7803.
[37] U. Neugebauer, Y. Pellegrin, M. Devocelle, R. Forster, W. Signac, N. Morand, T. Keyes, Chem. Commun. (2008) 5307–5309.
[38] N. O'Connor, N. Stevens, D. Samaroo, M. Solomon, A. Marti, J. Dyer, H. Vishwasrao, D. Akins, E. Kandele, N. Turro, Chem. Commun. (2009) 2640–2642.
[39] V. Fernandez-Moreira, F.L. Thorp-Greenwood, M.P. Coogan, Chem. Commun. 46 (2010) 186–202.
[40] R. Zhang, X. Yu, Z. Ye, G. Wang, W. Zhang, J. Yuan, Inorg. Chem. 49 (2010) 7898–7903.
[41] B.P. Sullivan, D.J. Salmon, T.J. Meyer, Inorg. Chem. 17 (1978) 3334–3341.
[42] P. Kus, G. Knerr, L. Czuchajowski, J. Heterocycl. Chem. 27 (1990) 1161–1166.
[43] L.D. Ciana, W.J. Dressick, A.V. Zelewsky, J. Heterocycl. Chem. 27 (1990) 163–165.
[44] G.A. Crosby, W.H.J. Elfring, Phys. Chem. 80 (1976) 2206–2211.
[45] I.K. Shoeb, E.B. Amy, D.S. Gregory, S. Milan, W.G. Mark, Inorg. Chem. 38 (1999) 2411–2415.